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Bacterial Filament Systems: Toward Understanding Their Emergent Behavior and Cellular Functions*

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Ye-Jin Eun‡§1,2,3, Mrinal Kapoor‡§1, Saman Hussain‡§3, and Ethan C. Garner‡§4

From the ‡Molecular and Cellular Biology Department and §Faculty of Arts and Sciences (FAS) Center for Systems Biology, Harvard University, Cambridge, Massachusetts 02138

Bacteria use homologs of eukaryotic cytoskeletal filaments to conduct many different tasks, controlling cell shape, division, and DNA segregation. These filaments, combined with factors that regulate their polymerization, create emergent self-organizing machines. Here, we summarize the current understanding of the assembly of these polymers and their spatial regulation by accessory factors, framing them in the context of being dynamical systems. We highlight how comparing the *in vivo* dynamics of the filaments with those measured *in vitro* has provided insight into the regulation, emergent behavior, and cellular functions of these polymeric systems.

The use of fluorescent proteins changed our view of the bacterial cell; rather than being homogeneous mixtures, bacterial cells are spatially organized, placing components at specific locations. With the discovery that bacteria contain structural homologs of eukaryotic actin and tubulin (1, 2), it was proposed that these polymers might have organizational and structural roles similar to their eukaryotic counterparts (3, 4).

The first homologs of the eukaryotic cytoskeleton discovered in bacteria are some of the most conserved. FtsZ is a tubulin that localizes to the cleavage plane and is essential for cell division (5). MreB is an actin distributed along the length of the cell, and it is essential in many bacteria for both rod shape maintenance and elongation (6). Although these two proteins have less than 15% sequence identity to their eukaryotic counterparts, they show a high conservation of tertiary structure (2, 7), and also hydrolyze and polymerize with the same nucleotide: FtsZ with GTP, and MreB with ATP (2, 8).

Filaments are also encoded by extra-chromosomal elements; low copy plasmids use polymers to bias segregation to both daughter cells. Examples include actins such as ParM found on *Escherichia coli* plasmids (9), AlfA on *Bacillus subtilis* plasmids (10), and many others (11). Additionally, tubulin homologs, such as TubZ, have been found on *Bacillus* plasmids (12). Even bacteriophages use tubulin filaments, such as PhuZ, to organize nascent phage particles to the host mid-cell (13).

We Are All Snowflakes: The Diversity of Bacterial Polymers

Ultrastructural studies of these distant homologs revealed that the actin and tubulin folds are capable of assembling into a wide variety of filaments. ParM forms two-stranded left-handed filaments (14). AlfA forms large bundles composed of short, mixed polarity, left-handed filaments (15). Unlike all other actins, MreB forms flat, antiparallel protofilaments, with laterally adjacent monomers in register, rather than staggered (16).

Among tubulins, FtsZ forms short, single-stranded filaments (17) that are straight or curved depending on conditions (18), whereas PhuZ forms three-stranded filaments with a right-handed twist (19). Interestingly, TubZ initially forms right-handed double filaments that transition into a four-stranded form following GTP hydrolysis (20).

In addition to the filaments listed above, there are many other families of bacterial filaments (extensively reviewed elsewhere (21)), including bactofilins, intermediate filament proteins, and metabolic enzymes. Collectively, these bacterial polymers have been termed the "prokaryotic cytoskeleton" (2, 21), a phrase historically based on the structural homology of FtsZ and MreB to the eukaryotic actin and tubulin folds.

This review summarizes the current understanding of how these polymers behave in isolation, and also how they are spatially regulated by other factors to create emergent biological machines. We highlight how comparisons of polymer dynamics *in vitro* and *in vivo* have shed light on both the regulation and the cellular function of these filaments. We start by reviewing "case studies" of the minimal plasmid segregation systems and detail how the same approaches have been used to study FtsZ and MreB.

Easy to Understand because There Are Only Three Things to Look at

Plasmid segregation systems contain only three components; the dynamic properties of their filaments both in isolation and in combination with the other two components have been well characterized. These systems are single operons, encoding a polymer, a DNA-binding protein, and a centromeric DNA sequence. From these parts, *ParMRC* builds two structures: ParM filaments and a ring-like kinetochore created by ParR binding to sequence repeats in *parC* (22, 23). Two pieces of *in vivo* and *in vitro* data suggested that ParR/*parC* modulates ParM dynamics to make a plasmid-segregating spindle. *In vivo*, long ParM filaments extend through the length of the cell with

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[3] Supported by the Howard Hughes Medical Institute.

[4] To whom correspondence should be addressed: Dept. of Molecular and Cellular Biology, FAS Center for Systems Biology, Northwest Lab Bldg. 445.20, 52 Oxford St., Cambridge, MA 02138. E-mail: egarner@g.harvard.edu.

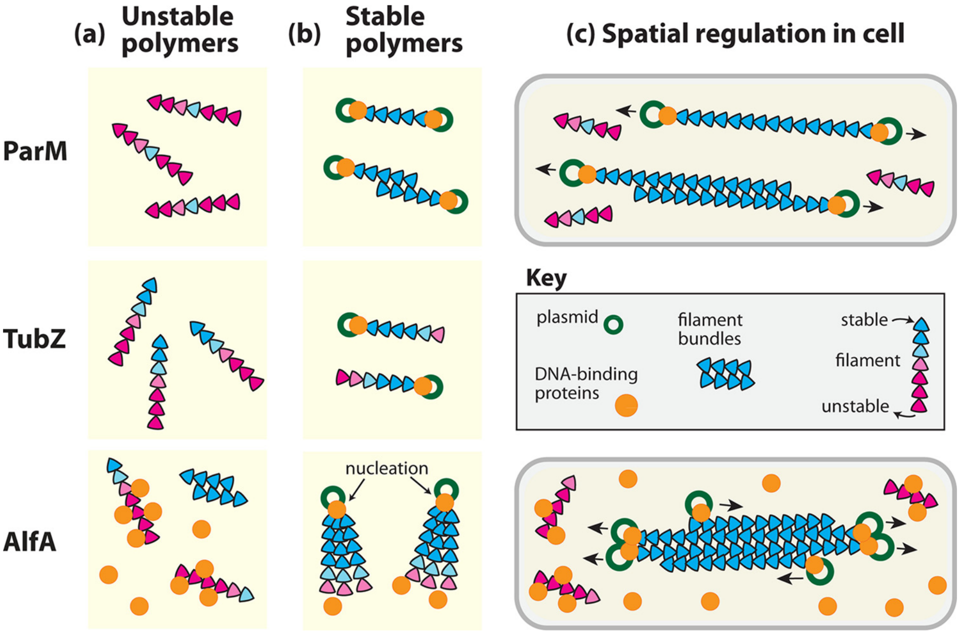

FIGURE 1. **Summary of three-component plasmid segregation systems.** A common theme among the ParM, AlfA, and TubZ spindles is the spatial regulation of polymer stability. *a*, the polymers have differing degrees of inherent instability (stable in *blue*, unstable in *red*). ParM is dynamically unstable, TubZ treadmills, and AlfA is stable over minutes, although it is destabilized by free AlfB. *b*, polymers within the spindle are stabilized in two ways: 1) interactions with the DNA-binding protein and plasmid DNA, and 2) lateral interactions with neighboring filaments. *c*, the native properties of each polymer combined with the regulation conferred by the other factors cause filaments in the spindle to be stable relative to those elsewhere in the cell, which favors filament elongation at kinetochores. The *black arrows* depict the direction of traveling plasmids.

plasmids at each end. Filaments were visible only when there was more than one plasmid (24). In contrast, ParM creates short, transient polymers *in vitro*. Assembly proceeds by rapid nucleation followed by growth from both ends. Filaments depolymerize from either end via hydrolysis-mediated dynamic instability (25). The discrepancy between the long, stable filaments *in vivo* and the short, unstable filaments *in vitro* was explained by combining all three components, which showed that ParR/*parC* stabilizes ParM filaments to the ATP state, inhibiting filament catastrophe at bound ends (26). This suggested that filaments, stabilized at both ends, elongate by insertional polymerization (26) (Fig. 1, top spindles in *b* and *c*). The turnover of unattached filaments provides free monomers to favor growth at ParR/*parC*. A later study found that interactions between ParM filaments can also stabilize them (27), suggesting that two filaments, each attached to a single kinetochore, can form a productive spindle, elongating only at the ParR/*parC*-bound ends (Fig. 1, bottom spindles in *b* and *c*). This study also observed that ParR/*parC* can bias the growth of ParM filaments assembled in AMPPNP[5] to the ParR/*parC*-bound end, a behavior noted in previous work (26). This biased growth led the authors to a model where 1) ParR/*parC* can bind to only one end of ParM filaments, and 2) like eukaryotic formins, ParR/*parC* increases the monomer on-rate at the bound end. However, this model creates a kinetic dilemma. ParR/*parC* accelerates polymerization of AMPPNP filaments only at the bound end (27), yet ATP ParM filament ends elongate at the same rate (at both ends) both when they are free and when they are ParR/*parC*-bound (25, 26, 28). This rate of ATP filament elongation matches the rate of spindle elongation observed *in vivo* (29). This kinetic discrepancy (which likely arises due to the difference in free monomer concentrations between the nucleotide conditions) remains to be resolved.

The *TubZRC* system is remarkably similar to *ParMRC*, despite using a tubulin. First, the TubR/*tubC* kinetochore structure strongly resembles ParR/*parC* (30). Second, TubZ polymers treadmill *in vivo* (31), indicating that they are also unstable, although less so than ParM. Third, TubR/*tubC* stabilizes polymers, allowing TubZ filaments to form beneath their normal critical concentration (30) (Fig. 1).

The Alf system uses alternative means to achieve the same goal. It comprises the actin AlfA, the DNA-binding AlfB, and the *parN* centromere. *In vivo*, AlfA forms large filament bundles that recover from photobleaching from both ends of the bleached region (10). However, bundles formed *in vitro* are stable over several minutes (15). This difference can be explained by the opposing effects of AlfB on AlfA. Free AlfB binds to the sides of AlfA filaments, inhibiting bundling and increasing filament turnover. In contrast, AlfB complexed with *parN* nucleates AlfA filaments, which form stable bundles via lateral interactions. AlfB/*parN* also lowers the critical concentration of AlfA, favoring growth at *parN*. Reconstitution of all three components *in vitro* demonstrated that AlfB/*parN* complexes ride on the ends of elongating bundles and also move along existing bundles in both directions. Continued growth at AlfB/*parN* is ensured by free AlfB repressing AlfA polymerization elsewhere in the cell (32) (Fig. 1).

In all the above cases, nucleation of these polymers by themselves is rapid and appears unregulated in space. Although the inherent polymer dynamics and regulation thereof vary greatly between these systems, they follow a

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[5] The abbreviation used is: AMPPNP, Adenylyl imidodiphosphate.

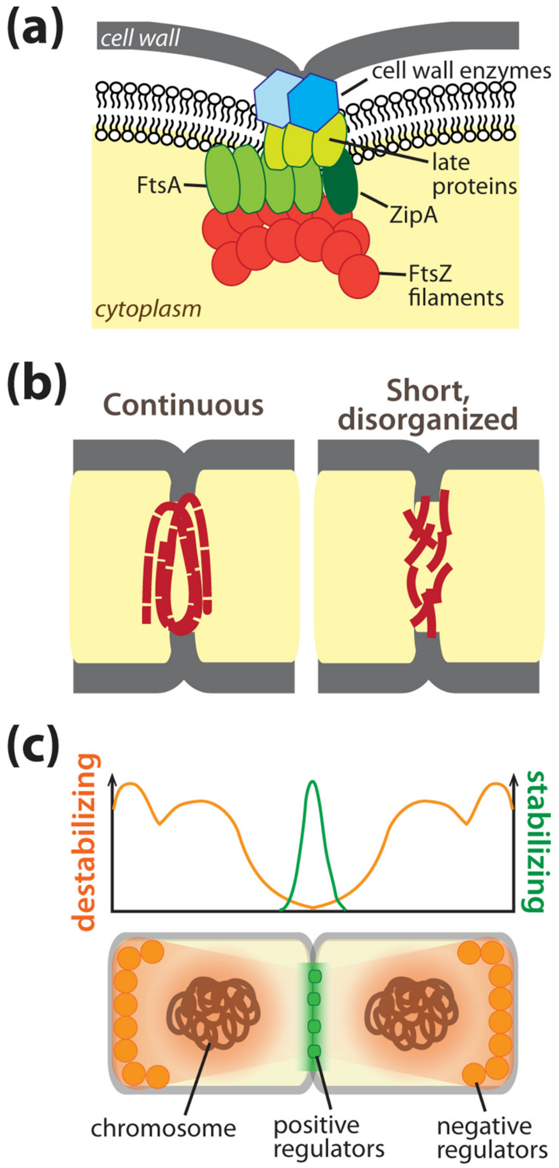

FIGURE 2. *a*, schematic of proteins at the division site. FtsZ polymers are anchored to the membrane through early divisome proteins (*e.g.* ZipA and FtsA). Other early proteins (*e.g.* ZapA and FzlA) that stabilize the Z-ring are not shown. Subsequently, late proteins (*e.g.* FtsKQLNBIW), including cell wall synthesis enzymes, arrive to continue divisome assembly. *b*, two models of Z-ring structure. The first model (*left*) suggests that FtsZ filaments form a continuous ring or compressed helix. The second model (*right*) proposes that the ring is made of short, disorganized polymers with a few lateral contacts. *c*, spatial regulation of FtsZ polymerization in the cell. Both positive and negative regulators are spatially organized in the cell to focus the Z-ring. The concentrations of regulators along the cell length are plotted, showing that destabilizing factors are located at the poles and over the chromosome, whereas stabilizing factors are at the division plane. This schematic reflects the overall spatial organization in *E. coli* and *C. crescentus*.

common principle: The subset of filaments attached to the kinetochore is stabilized relative to free filaments. This allows the stable spindle to elongate using the monomer pool provided by the turnover of unattached filaments (Fig. 1). We wish to note that it is possible that nucleation may also occur at the kinetochores of all these systems. Although AlfA is the only bacterial polymer found to be regulated by nucleation, it has not been proven that the other polymers are also nucleated at the plasmids.

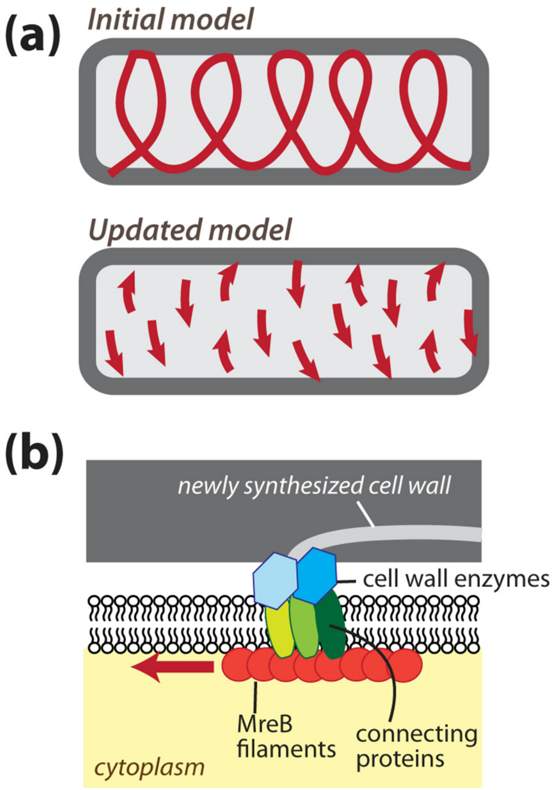

FIGURE 3. *a*, models of MreB polymer organization *in vivo*. The initial model proposed that MreB polymers make up a cell-spanning helix. The updated model suggests that disconnected polymers move circumferentially around the rod-shaped cell; their motions are otherwise uncoordinated. Moving MreB is shown as *arrows*. *b*, schematic of proteins involved in elongation. MreB filaments bind to the membrane and interact with peptidoglycan synthesis enzymes in the periplasm through transmembrane connector proteins. Cell wall synthesis drives MreB motion, indicated by the *red arrow*.

Dear Eukaryotic Cytoskeleton: I May Look Like You, but I'm Different

Unlike the eukaryotic cytoskeleton, FtsZ and MreB do not directly impose cellular geometry themselves; they influence bacterial cell wall synthesis, a complex process involving many proteins (33). FtsZ and MreB in the cytoplasm control the activity of the cell wall-synthesizing enzymes on the opposite face of the membrane, defining the cellular location of cell wall synthesis and thus the shape of the cell (Figs. 2*a* and 3*b*). Therefore, to understand bacterial growth and division, we must understand how these filaments and their accessory factors function as complete systems.

FtsZ: The Closer We Look, the Weirder It Gets

FtsZ is required for localization of all other proteins that form the "divisome" (34) (Fig. 2*a*). Divisome proteins assemble in a hierarchical, two-step fashion: "early" and "late." Early proteins tether FtsZ to the membrane and regulate its polymerization and structure. Late proteins synthesize the cell wall and segregate any chromosomes trapped at the constriction (34) (Fig. 2*a*).

In vivo, FtsZ can form multiple structures: a tight ring at the mid-cell (i.e. Z-ring) and shorter spirals or arcs near the division site (35). FtsZ was reported to form long helices spanning the cell length (36), but these structures are likely artifacts caused by conditions that increase polymer formation (37); they appear when the cellular FtsZ levels are increased or when the rate of GTP hydrolysis is reduced (38, 39). It is currently thought that the Z-ring represents a productive divisome and that the shorter spirals/arcs may be less stable Z-rings or intermediates (40).

Filament organization within the Z-ring is still under debate (Fig. 2*b*). Two contrasting models have resulted from multiple studies using super-resolution microscopy and cryo-EM. One model suggests that FtsZ forms a continuous ring of connected filaments (39, 41). The other model holds that the Z-ring is composed of short (50–500 nm) filaments that are connected by a few lateral contacts and are irregularly distributed around the division plane (42–44).

Z-rings are very dynamic, and their turnover is regulated by GTP hydrolysis (45, 46). The halftime for turnover of filaments *in vivo* (8–11 s) is similar to that measured for filaments *in vitro* (3.5–7 s) (40). Single molecule studies suggest that FtsZ monomers within the Z-ring are frozen in position for at least 3–4 s (47). Combined, these results imply that each monomer is immobile for a substantial fraction of its lifetime in the Z-ring.

How Accessory Factors Focus the Z-ring in Space

In vitro, FtsZ rapidly forms single-stranded filaments, 30–50 subunits long (40). Although these properties seem to indicate that FtsZ assembly is isodesmic (17), polymerization is in fact cooperative; FtsZ monomers are first "activated" via GDP release and subsequently dimerize to form a nucleus for polymerization (48). These results indicate that FtsZ filaments alone should polymerize everywhere throughout the cytoplasm. Instead, cells create a precisely positioned and stable ring at the membrane, indicating that the stability of FtsZ filaments must be spatially regulated in the cell.

Bacteria position their Z-ring with remarkable precision, within an error of 2.9% of the cell length in *E. coli* (49). A properly positioned Z-ring must fulfill two criteria. It should not close around the nucleoid, and it should be positioned at the mid-cell. Both tasks are accomplished by negative regulation of polymerization. To avoid the nucleoid, bacteria use DNA-binding proteins, such as Noc and SlmA, that locally inhibit filament formation (50) (Fig. 2*c*); Noc associates with both DNA and the membrane to physically block Z-ring formation. SlmA antagonizes FtsZ polymerization, although its exact mechanism of inhibition remains controversial (50).

For the second task of finding the middle of the cell, cells create spatial gradients of depolymerizing factors: maximal at the poles, minimal at mid-cell (Fig. 2*c*). These gradients can be positioned in different ways. In *Caulobacter crescentus*, the FtsZ inhibitor MipZ associates with ParB, which binds near the origin of replication on the chromosome. Before replication, MipZ is held only at one cell pole (51). As the DNA replicates, one origin segregates to the opposing pole, whereas the other remains in place. Positioning the MipZ maxima at poles creates a bipolar gradient minimal at the mid-cell, where the Z-ring forms (51). In contrast, in *E. coli*, the gradient establishes itself using a reaction-diffusion system (52). This system has three components. MinD is an ATPase that self-assembles on the membrane and also binds the FtsZ depolymerizer MinC. MinE promotes ATP hydrolysis by MinD, causing it to detach from the membrane. Together, these activities cause all three components to oscillate between cell poles, creating a time-averaged minimum of MinC at mid-cell (53).

Reconstitutions of MinCDE and FtsZ in three-dimensional cell-shaped compartments demonstrated that MinCDE together form a time-averaged bipolar gradient, confining FtsZ filaments to the middle region. Interestingly, this biochemical system responds to its surrounding geometry, creating multiple MinC minima in compartments of varying lengths (54). These experiments show another result. Although confined, the filaments within these minima do not form a focused Z-ring, indicating that positive regulation is required for Z-ring formation.

Many proteins are known to stabilize FtsZ filaments (Fig. 2*c*). FtsA, which itself can form filaments (55), is one of the few proteins known to bring FtsZ to the membrane. *In vitro* reconstitutions have shown two effects of FtsA. First, it co-assembles with FtsZ polymers onto membranes. Second, shortly after co-assembly, FtsA destabilizes FtsZ, causing monomers to detach from the filaments (56). These time-delayed properties cause FtsZ filaments to "move" in linear paths by treadmilling along the membrane as monomers remain immobile with respect to the treadmilling filament (56).

Other membrane proteins, such as ZipA and ZapA, positively regulate FtsZ polymerization by reducing the rate of filament turnover. ZipA accomplishes this by promoting FtsZ bundling, as shown by reconstitution on lipid bilayers (56). In another study, ZapA-mediated bundling protected FtsZ from the depolymerizing effects of MinC (57).

Other proteins modulate the superstructure of FtsZ filaments. FzlA bends *C. crescentus* FtsZ into helical bundles, and in doing so, might contribute to the constriction of the septum (58). In *B. subtilis*, SepF makes rings and arcs that wrap around FtsZ filaments (59), perhaps further anchoring FtsZ to the membrane (60).

We are just beginning to explore the effects of each individual divisome protein on FtsZ filaments. Future *in vitro* studies may reveal more complex behaviors when multiple regulators are combined. Deconvoluting the complexity of the divisome may be assisted by studying FtsZ dynamics *in vivo* in the absence of each factor; this could also be done at different stages of divisome maturation and Z-ring constriction.

How Does the Z-ring Divide the Cell?

It is not known which component of the cell division machinery exerts the force required for division, but many models propose that it is FtsZ. Some models do not require a continuous Z-ring around the cell.

One early suggestion was that a change in FtsZ filament curvature upon GTP hydrolysis could pinch the cell (61). To test whether this occurs *in vitro*, FtsZ was fused to a membrane targeting sequence and encapsulated inside lipid tubes (62). FtsZ rings formed within these tubes could slide along their length. When sliding rings coalesced, they slightly constricted the tubes (62). However, follow-up studies cast doubt on this model because membrane deformations occurred even when FtsZ could not hydrolyze GTP (63, 64).

A second model proposes that FtsZ filaments are always more curved than the membrane they bind to, regardless of their nucleotide state. The repeated attachment of these short, bent filaments would deform the membrane, and the iterative local pinches could be coordinated by lateral interactions into a cell-scale constriction. This model was supported by studies showing that attachment of an artificial amphipathic helix to either side of the bent filament could deform liposomes in opposite directions (63).

A third model is that the ring constricts by contiguous filaments sliding past each other, tightening like a coiling spring (65, 66). This model relies on the idea that the Z-ring is composed of long, continuous filaments. The energy driving constriction arises from increasing the number of lateral interactions as the rings further condense (65, 66). This model was supported by a recent cryo-EM study showing continuous rings of FtsA/FtsZ filaments going around the entire septa of both *E. coli* and *C. crescentus* (41). Additionally, FtsA and FtsZ contained inside liposomes co-polymerized into filaments that encircled the inner face of the membrane. These filaments laterally associated, constricting the liposomes. The authors proposed that membrane constriction is initiated by the formation of a closed Z-ring and is continued by filaments sliding along with ongoing FtsA/FtsZ co-polymerization.

A fourth, divergent model proposes that FtsZ filaments do not exert force, but simply serve as a scaffold to localize the cell wall enzymes to the septum. The enzymes could then orient their synthesis inward to divide the cell. Although this model is difficult to test, cell wall synthesis is required for cell division as its inhibition via mutagenesis or antibiotics leads to incomplete constriction (67). Furthermore, depletion of an endopeptidase (*dipM*) in *C. crescentus* causes pre-existing constrictions to relax, whereas FtsZ still remains at the division site (68).

The two most frequently discussed models are 1) local bending by short filaments and 2) filament sliding within a continuous ring. When considered individually at its conceptual extreme, each model can be easily invalidated. For example, recent work has suggested that FtsZ filaments may be too flexible to bend membranes except at low surface tensions (69), calling into question the local bending model. Evaluating the other extreme, if filaments slide past each other, monomers should move across the septum, yet *in vivo* they are immobile (47).

These models need not be exclusive as the physical principles underlying each model may work together to divide the cell. The basis of the bending model is that membranes deform to match filament curvature. The basis of the sliding model is that increasing the lateral interactions induces constrictions (70). It has recently been shown that local crowding of any membrane protein, even GFP, can induce membrane deformation (71). These two forces may work together. Membrane constriction via local bending could be reinforced by lateral associations that further deform the membrane.

There is a remaining "energetic difficulty" with the filament sliding model (40). To add even one more lateral bond, the filaments must break *all* existing lateral ones as they slide relative to each other. We suggest that this difficulty may be resolved by the recent observation of FtsA/FtsZ treadmilling (56). This may reduce the energetic barrier for filaments to move relative to each other, increasing contacts via new growth without breaking existing bonds.

We also suggest that a similar, multi-model view can be taken regarding the filament structure within the Z-ring. It is possible that different filament organizations exist at each stage of divisome maturation and constriction.

MreB, the Bacterial Problem Child

Although MreB has been extensively studied, we know very little about the dynamics and cellular function of MreB filaments. In concert with a small group of other proteins, MreB both encodes and maintains the width (*i.e.* diameter) of rod-shaped bacteria as they elongate (72). Initial *in vivo* observations painted a picture of MreB that persisted for a decade. MreB was seen to form long, cell-spanning helices (6, 73) (Fig. 3*a*). These helices were proposed to localize the cell wall synthesis enzymes, directing a helical pattern of cell wall growth (74).

Although the overall MreB helices appeared mostly static in Gram-negative bacteria, single molecule approaches in *C. crescentus* revealed that MreB monomers move linearly in both directions around the cell, traveling a short distance before dissociating (75). These motions, at the time, were attributed to monomers treadmilling through short filaments within a static helix. In *B. subtilis*, entire filaments were reported to move in helical tracks around the cell, a motion also attributed to polymer dynamics (76).

The observation of cell-spanning structures created by this actin homolog suggested that it might have cytoskeletal functions as well as a role in cell wall synthesis. It was proposed that MreB, like actin, could function as a polymerization motor to transport cargo (4) or segregate newly replicated origins to the cell poles (77). MreB was also suggested to organize not only the proteins involved in cell wall synthesis, but many other unrelated proteins as well, as evidenced by co-localization and protein interaction studies (reviewed in Ref. 78).

Although MreB is notoriously difficult to work with *in vitro*, its assembly dynamics presented two features distinct from actin. Both its nucleation and its polymerization are rapid (79, 80), first forming short filaments that later coalesce into large bundles (81).

A series of recent findings has prompted a re-evaluation of our understanding of nearly every aspect of MreB: 1) its biochemical properties and assembly kinetics, 2) its *in vivo* structure and dynamics, and 3) its roles in the cell. This calls into question whether any analogies can be made between this prokaryotic filament and its eukaryotic doppelgänger.

First, it was discovered that MreB polymerizes on membranes, forming short, antiparallel filaments, rather than the bundles that form in solution (16, 82). Not only does this finding explain why previous *in vitro* studies were stymied by protein aggregation (83), it also indicates that the results from these initial kinetic studies need to be reassessed.

Next, the "cell-spanning helix" was found to be an artifact. In *E. coli*, N-terminal fluorescent fusions gave rise to the static

MreB helices, which were revealed to be large bundles when imaged by cryo-EM (84). When the fluorophore was moved into an internal loop of MreB, no bundles were observed, and filaments could be seen to move across the cell (85). Further studies of MreB motion in *B. subtilis* indicated that MreB is a series of disconnected filaments that move radially around the cell circumference (86–88). These circumferential motions were found to be driven not by polymer dynamics, but by cell wall synthesis; they halt upon chemical inhibition or genetic depletion of the cell wall synthesis enzymes (85–87), which themselves move around the cell in the same manner as MreB (86, 87) (Fig. 3*a*).

Different lengths of MreB filaments *in vivo* have been reported. One study described small "patches" (87) in *B. subtilis*, whereas another reported filaments up to 3 μm long (88). These differences in length may arise from the expression level of MreB, or perhaps from the oligomerization caused by fluorescent fusions (see below). Although the length of MreB filaments remains controversial, it has been shown that MreB filaments are disconnected. Two different studies indicated no coordination between filament motions along the cell length at any resolvable scale (86, 89) (Fig. 3*a*).

Combined, these findings necessitate a reappraisal of the roles of MreB in the cell. As MreB forms short (relative to the cell length), uncoordinated filaments that move around the cell width, MreB must create shape locally, rather than at the cell-length scale. Furthermore, the other proposed cellular functions of MreB require re-evaluation. 1) Such filaments cannot spatially organize other cellular components over long distances, and 2) their polymerization cannot be harnessed to move cargo in the cell as they are extremely stable (87) and lack structural polarity (a feature that rules out directed filament growth) (16), and their cellular motions are driven by cell wall synthesis, not by polymerization (86).

Regulation of MreB

As is the case with most biological filaments, the polymerization of MreB appears to be regulated. In *B. subtilis*, MreB filament association with the membrane is regulated via Lipid II, the lipid-linked precursor for cell wall synthesis. If Lipid II levels are decreased, filaments detach from the membrane (90). Although the factors mediating this process (or any other regulation) have not been identified, there are many candidates given the long list of proteins reported to be associated with MreB (78). However, many of these interactions were assayed using fluorescence co-localization, often with the artifactual MreB helix, and thus warrant re-examination. Furthermore, it was found that MreB is a common contaminant in affinity pulldowns (91), indicating that many interactions identified by immunoprecipitation or affinity tags may also be suspect.

Like the divisome and FtsZ, proteins that interact with MreB, including MreC, MreD, RodA, and RodZ (78), may regulate filament levels. Of these, RodZ is an appealing regulatory candidate because its cytoplasmic domain has been crystallized with MreB (92), and expression of this domain alone can partially restore cell shape and MreB localization in *rodZ*-null cells (93). Other candidates have been found, but require further study. These include DapI, a cell wall synthesis enzyme in *B. subtilis* that interacts with cytoplasmic MreB (91), and MbiA, a protein in *C. crescentus* that affects cell shape by interacting with MreB, although its biological function remains unknown (94).

How Does MreB Define Cell Width?

It remains a mystery how this disconnected system of rotating filaments imparts a robust width to rod-shaped cells. These filaments influence the insertion of cell wall locally, yet uniformly, over long distances, giving a constant radius along the cell length.

Some studies have used MreB mutants to approach this question. By examining *C. crescentus* with MreB mutated near the ATP-binding site, Dye *et al.* (95) found that alterations in MreB polymerization dynamics affect its cellular distribution, which then affects the shape of the cell. One of these MreB mutants created irregularly shaped cells, fat in some areas and very thin in others. This mutant MreB preferentially localized to the thinner regions of the cell, causing those areas to elongate while the other regions bulged (96). This led to the proposal that MreB filaments serve to maintain the cell radius, not to encode it.

Any bends or defects in a growing rod-shaped bacterium need to be straightened out. Two different studies have found that MreB filaments localize to regions of negative curvature, which are small "inward dimples" along the cell length (97), and the inner (more curved) face of a sharply bent cell (98). Thus, the MreB-mediated insertion of new cell wall material at these areas would flatten out small local defects, or straighten bent cells.

The Filaments Are Short, Disconnected, and Mobile. How Is That a "Skeleton"?

Although actin and tubulin homologs are used by eukaryotes and prokaryotes to accomplish similar tasks (*e.g.* cell shape maintenance, cell division, and DNA segregation), they differ in their filament structure, assembly kinetics, and spatial regulation. Even among the prokaryotic filaments, there is a wide diversity of properties, demonstrating that biology can solve similar problems using many different strategies.

Eukaryotic and prokaryotic filaments also differ in the length scales they coordinate. The eukaryotic cytoskeleton is a system of interconnected fibers: a mechanically coherent structure that is persistent over long distances, setting up a global cellular coordinate system. In contrast, the most conserved bacterial filaments of the prokaryotic cytoskeleton, MreB and FtsZ, confer cell shape by locally influencing cell wall synthesis. These filaments are short and disconnected (debated for FtsZ), and thus cannot globally organize the cell. Moreover, recent studies showed that protein localization patterns could be dramatically altered by the fluorescent tag used (99, 100). In one study, constructs with 14 different fluorescent proteins fused to a common target (ClpXP) were compared, demonstrating that some fusions induce clustering of soluble proteins. Given these worrisome findings, it will be interesting to see how organized bacterial cells really are.

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